occurred (Table 1, I). Masking, moreover, did not affect the known primary attraction to host substances (Table 1, J_1 and J_2).

It is clear that masking is triggered by stridulation of the male in proximity to the attractive female. Presumably the sound of the stridulation is received by a phonoreceptor of the female, and so the release of the masking substance is controlled by the central nervous system. This phenomenon indicates the interplay of possible messengers, as both auditory and olfactory stimuli are present.

Although the mask stopped the attraction of flying beetles, the arrested males whose stridulation triggered the mask were observed to remain at the screened entry holes for at least 4 days after the mask occurred. It is possible that the continuously fresh frass, produced as the female constructs the gallery, is sufficiently attractive to hold the arrested males there. However, it seems more likely that male arrestment with stridulation, or "calling," represents a critical stage in the secondary process of attraction of the Douglas-fir beetle. In other words, attraction of the beetles in flight can be stopped by masking, but not the premating behavior of the arrested males whose stridulation triggered the masking. Release of the masking substance may be considered part of the female mating behavior also; it occurs not only when stridulating males approach but also during the prolonged nudging movements that characteristically precede mating in this species and, of course, after mating. Perhaps this phenomenon, when investigated further, will shed some light on the complex interrelation of population aggregation and sex behavior in those bark beetles whose survival depends not only on finding a mate but also on mass attack of the host tree (*7*).

After the female Douglas-fir beetles reemerge from the bark and reattack new hosts (summer attack), they again produce the aggregating pheromone (*2*). Whether the mask also occurs during this second flight and aggregation was not investigated.

The survival value of the mask is clear. It tends to distribute evenly the available males, while both preventing overcrowding with resultant brood mortality and allowing the mass attack necessary to overcome host resistance (*6*). This phenomenon gives a new dimension to the already intricate mechanism of host and insect forms of attraction in this destructive bark beetle. It complicates our present effort to establish a control system by manipulating the aggregant pheromone, but it may ultimately offer a new possibility of control if saturation quantities of the masking substance can be used to prevent aggregation of the flying population to especially valuable or vulnerable stands of Douglas fir.

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Membranous Structures Associated with Translation and Transcription of Poliovirus RNA

Abstract. *Poliovirus RNA and proteins are synthesized in association with distinct membranous structures that were separated by means of isopycnic centrifugation of cytoplasmic extracts in discontinuous sucrose-density gradients. Viral RNA is replicated in a structure that contains rapidly labeled replicative intermediate RNA and viral RNA polymerase associated with the smooth membrane fraction. In sucrose gradients this viral RNA replication complex is distributed at densities in the range of 1.12 to 1.18 grams per cubic centimeter. Viral proteins are synthesized on polyribosomes bound to membranes and sediment with polyribosomes at densities of less than 1.25 grams per cubic centimeter.*

Host cell membranes are involved in the replication of picornaviruses, a group of small animal viruses free of lipids, which contain RNA as their genetic material. As shown by cell fractionation, the synthesis of both viral protein and viral RNA is associated with membranous structures in the cytoplasm of infected cells (*1–3*). Furthermore, proliferation of cellular membranes after virus infection has been detected by electron microscopy (*4, 5*) and by incorporation of radioactive choline (*6*).

Cytoplasmic membranes can be separated by a method of isopycnic centrifugation described by Bosman *et al.* (*7*). Using a modification of this technique, we have isolated the membranous structures associated with translation and transcription of poliovirus RNA. Our results indicate that each process is associated with a distinct membranous structure.

The S3 HeLa cells in suspension were infected with poliovirus, treated with actinomycin D, and incubated at 37°C as described (*8*). After harvest and washing, the cells were swollen in hypotonic buffer, RSB [0.01*M* tris(hydroxymethyl)aminomethane, *p*H 7.4, 0.01*M* KCl, and 0.0015*M* $MgCl_2$], for 20 minutes and then broken open with 15 strokes in a tight-fitting Dounce homogenizer. Nuclei and cell debris were removed by centrifugation at 900*g* for 10 minutes, and the supernatant cytoplasmic extract was made 30 percent (by weight) in sucrose by addition of 60 percent sucrose in RSB (by weight). A discontinuous gradient was formed by layering sucrose-RSB solutions in the following order: 3 ml of 60 percent sucrose, 7 ml of 45 percent sucrose, 7 ml of 40 percent sucrose, 10

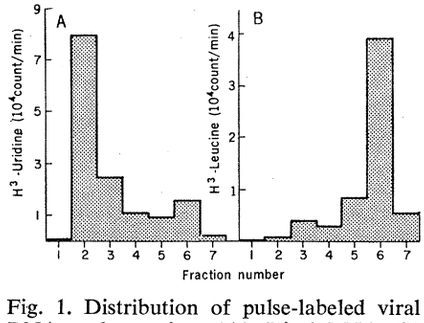

Fig. 1. Distribution of pulse-labeled viral RNA and proteins. (A) Viral RNA. S3 HeLa cells (4.1×10^8) were infected with poliovirus type 2 and treated with actinomycin D (5 μg/ml) as described (*8*). At 3.25 hours after infection the culture received a 2.5-minute pulse of uridine-5-^{3}H (20 μc/ml). Cytoplasmic extract was prepared and analyzed by means of isopycnic centrifugation through a discontinuous sucrose gradient. A portion of 0.2 ml from the pelleted material of each fraction was analyzed for trichloroacetic acid–precipitable radioactivity (*8*). (B) Viral protein. The same procedure as described in (A) was used except the culture received a 3-minute pulse of leucine-^{3}H (20 μc/ml).

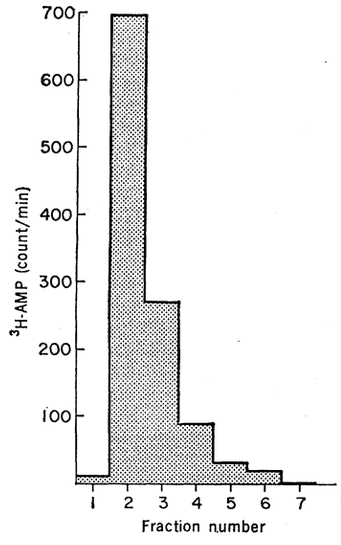

Fig. 2. Distribution of viral RNA polymerase activity. S3 HeLa cells (6.0×10^8) were infected, harvested 3.25 hours later, and fractionated as described in Fig. 1. Viral RNA polymerase activity of the pelleted material from each fraction was determined with 250 μc of tritiated adenosine triphosphate (ATP) as the labeled precursor (*11*). The results are expressed as counts per minute per microgram of protein.

ml of cytoplasmic extract containing 30 percent sucrose, 7 ml of 25 percent sucrose, and 3 ml of RSB on top. The gradients were centrifuged at 86,000*g* for 17 to 19 hours in a Spinco SW27 rotor. Seven unequal fractions, which contained visible bands of material, were collected by means of a Pasteur pipette (*9*). The first three fractions contain predominantly smooth membranes, whereas fractions 4 to 6 contain rough membranes. These results, obtained by electron microscopy and sedimentation of labeled ribosomes, are similar to the findings reported by Bosman *et al.* (*7*). The density of each fraction at 4°C is as follows: fraction 1, 1.079 g/cm^3; fraction 2, 1.119 g/cm^3; fraction 3, 1.179 g/cm^3; fraction 4, 1.206 g/cm^3; fraction 5, 1.246 g/cm^3; fraction 6, 1.265 g/cm^3; and fraction 7, 1.312 g/cm^3. The fractions banded at approximately the same density when centrifuged through sucrose gradients a second time.

After collection, each fraction was diluted to 11 ml with RSB and then centrifuged at 100,000*g* for 2 to 2.5 hours in a Spinco S40 rotor. The pellets were suspended in 2 ml of buffer by means of a Teflon pestle, and a portion from each pellet was assayed for chemical composition, radioactivity, infectivity, or ezyme activity.

The pattern of distribution of rapidly labeled viral RNA (Fig. 1A) differs markedly from that of viral protein (Fig. 1B). Most of the rapidly labeled virus-specific RNA is in fractions 2 and 3, with a small but significant amount in fraction 6 (Fig. 1A). In contrast, the peak amount of virus-specific proteins is in fraction 6; fractions 5 and 7 also contain significant amounts (Fig. 1B). Electron microscopy of fraction 2 reveals predominantly smooth membranes, whereas fraction 6 contains rough membranes with clusters of ribosomes or polysomes attached.

The pattern of distribution of viral RNA polymerase shows (Fig. 2) that almost all of this enzyme activity is in fractions 2 and 3, which also contain over 50 percent of the rapidly labeled RNA. The RNA extracted from fraction 2 is rich in the replicative intermediate species of viral RNA. These results indicate that the poliovirus RNA replication complex, which contains viral RNA polymerase and the replicative intermediate species of viral RNA (*10*), is associated with smooth cytoplasmic membranes.

Viral polyribosomes with a sedimentation coefficient of 350*S* are found in fraction 6 (*9*). In addition, almost all of the infective virus sediments in fractions 5 and 6 (Fig. 3). Our results suggest that the pulse-labeled viral protein sediments as nascent polypeptides attached to viral polyribosomes or as proteins incorporated into partially completed virus particles. It is unlikely that any of the protein labeled after a 3-minute pulse is in infective virus, as it takes about 20 minutes for newly synthesized viral protein to be incorporated into mature virus particles (*1*).

The membranous structures associated with translation and transcription of poliovirus RNA are apparently distinct and can be separated by means of isopycnic centrifugation in sucrose. Our results and the results of an electron microscopic study of poliovirus-infected cells (*4*) do not support the view that synthesis of poliovirus takes place in a single structure, a "virus synthesizing body," as was proposed and widely accepted earlier (*1*).

Our results support the following concept of cellular localization of transcription and translation of poliovirus RNA. Viral RNA replicates in a structure which sediments with smooth cytoplasmic membranes. This structure is detected by the presence of rapidly labeled replicative intermediate RNA and viral RNA polymerase activity, and is recovered in the upper fractions of a discontinuous sucrose gradient. Viral proteins are synthesized on polyribosomes which are bound to membranes and are distributed, along with infective virus particles, in higher density fractions in sucrose gradients.

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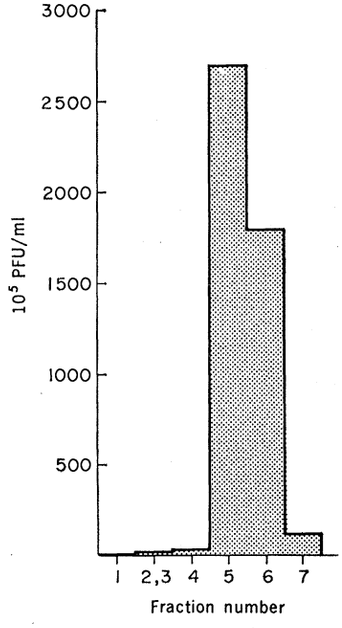

Fig. 3. Distribution of infective poliovirus. S3 HeLa cells (1×10^8) were infected, harvested 4 hours later, and fractionated as described in Fig. 1. Infectivity [plaque-forming units per milliliter (*PFU/ml*)] of the pelleted material from each fraction was determined in HeLa cell monolayers.

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